CANCER GENOMICS & PROTEOMICS 5: 333-340 (2008)

# Prognostic Utility of Glycosyltransferase Expression in Breast Cancer

NEILL PATANI[1], WEN JIANG[2] and KEFAH MOKBEL[1,3]

[1]*The London Breast Institute, The Princess Grace Hospital, London;*
[2]*Cardiff University, Cardiff, Wales;*
[3]*St. George's University of London, London, U.K.*

**Abstract.** *Background: The post-translational modification of proteins, including glycosylation, is known to differ between normal and tumour cells. In this study, the expression profile of two glycosyltranferases, UDP-N-acetyl-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase-6 (ppGalNAc-T6) and α6-sialyl-transferase-I (ST6GalNAc-I) was assessed, in a cohort of women with breast cancer. Patients and Methods: Breast cancer tissues (n=127) and normal background tissues (n=33) were collected immediately after excision during surgery. Following RNA extraction, reverse transcription was carried out and transcript levels were determined using real-time quantitative PCR and normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression. Transcript levels within the breast cancer specimens were compared to the normal background tissues and analyzed against conventional pathological parameters and clinical outcome over a 10 year follow-up period. Results: Significantly higher levels of ppGalNAc-T6 were found in the breast cancer specimens compared to the background tissue (p=0.015). There was a non-significant trend for levels to increase with the Nottingham Prognostic Index (NPI) and TNM stage and those who died from breast cancer. ST6GalNAc-I expression was associated with better prognosis, reaching significance when comparing patients who remained disease free to those with distant recurrence (p=0.0096). The relationship approached significance when comparing NPI 2 to NPI 3 (p=0.058) and disease free patients to non-disease free patients (p=0.052) or those who died of breast cancer (p=0.060). For both enzymes a significant association with ductal type was found. Conclusion: Expression of ppGalNAc-T6 is significantly higher in breast cancer compared to 'normal'/benign breast tissue samples. ST6GalNAc-I expression in breast cancer is associated with better prognosis.*

*Correspondence to:* Prof. K. Mokbel, MS FRCS, London Breast Institute, The Princess Grace Hospital, 45 Nottingham Place, London, W1U 5NY, U.K. Tel: +44 0 207 908 2040, Fax: +44 0 207 908 2275, e-mail: kefahmokbel@hotmail.com

*Key Words:* Breast cancer, glycosylation, prognosis, survival, recurrence.

Our appreciation of the extent to which tumour cells differ from their normal counterparts has developed significantly over the last two decades, driven largely by technological improvements in analytical techniques. Genetic abnormalities have been shown to contribute to the malignant phenotype through their attendant transcriptional and translational consequences. More recently, advances in molecular biology have permitted the study of post-translational events, such as glycosylation, which have the potential to significantly modify protein structure. Such alterations can result in the expression of a variety of tumour-associated carbohydrate antigens (1). The glycoproteins of tumour cells, including secreted or cell-membrane associated mucins, show significant variation, particularly within the mucin-type O-glycans, which harbour several cancer associated epitopes including the Thomsen-Friedenreich (T) antigen, Thomsen-nouveau (Tn) antigen, sialyl-Tn (STn) antigen and certain Lewis antigens (2, 3). In addition to conferring structural changes, patterns of glycosylation can have significant and diverse implications for glycoprotein function, including: signal transduction, antigenicity and interactions with immune effector cells, cell-cell and cell-stroma adhesion, angiogenesis, invasive potential and the metastatic competence of tumour cells (4-8). Hence, the study of such post-translational modifications could provide valuable mechanistic insights, presenting opportunities for diagnostic, prognostic and potentially therapeutic applications (9-11).

The complex and subtle molecular mechanisms which influence cellular glycodynamics remain poorly understood. Glycosylation patterns are influenced by a family of glycosyltransferase enzymes, whose specificities, sequential action, relative activity levels and intracellular localization are of critical importance in determining a cell-specific O-glycosylation profile (12). Mucin type O-glycosylation begins

with the addition of GalNAc to serine and threonine amino acids on the polypeptide by UDP-N-acetyl-D-galactosamine: polypeptide N-acetylgalactosaminyltransferases (ppGalNAc-T's) which are localised throughout the golgi (13, 14). The resulting antigen, termed the Tn antigen, is then modified by the addition of sugar residues. This process is catalysed by further glycosyltransferases, through systems referred to as the core 1 or core 2 pathways, in order to generate a variety of O-glycans (15-17). Under normal conditions, the Tn moiety is then masked by subsequent sugar residues. The differential expression of ppGalNAc-T's in cancer cells could lead to increased initiation of O-linked glycosylation with normally unoccupied potential glycosylation sites being glycosylated and cancer-associated antigens, such as Tn, emerging on the cell surface (18-21). Pathological exposure of such core region carbohydrates in cancer may reflect the deregulation of mechanisms responsible for the synthesis of the Tn antigen, or equally important, those involved with its further processing.

The ppGalNAc-T enzyme family has been implicated in the aberrant glycodynamics of several neoplasms (1). Each isoform has a different substrate specificity and expression profile. Expression and distribution studies have found the sixth member of the ppGalNAc-T family, ppGalNAc-T6, to be very restricted in normal tissue, but present in oral squamous carcinomas (22, 23). Although completely absent in healthy breast tissue, the enzyme has a high degree of homology with ppGalNAc-T3, the mRNA of which has been identified in cell lines derived from mammary gland adenocarcinomas (24). Freire *et al.* (25) concluded that ppGalNAc-T6 showed the greatest specificity for breast cancer, as confirmed by Brooks *et al.* (26).

In breast carcinomas there is a tendency for the addition of shorter O-glycans (27), which are themselves associated with increased sialylation (28), resulting in the overexpression of sialylated antigens at the surface of cancer cells. The sialyltransferases represent a group of enzymes which catalyze the biosynthesis of sialylated glycans (29). One potential substrate for this group of enzymes is the Tn antigen. Sialic acid is added in α2-6 linkage to GalNAc by α6-sialyltransferase (ST6GalNAc) (30). Of the two isoforms of this enzyme, α6-sialyltransferase I (ST6GalNAc-I) is believed to be the principal STn synthase *in vivo* (31). The carbohydrate antigen STn is a tumour associated disaccharide carried by mucins or within mucin like domains of several glycoproteins, including cell surface associated mucin 1 (MUC1), cluster differentiation 44 (CD44) and integrin subunits (32-35). STn expression is normally restricted to the lumen of secretory tissues and is absent from the breast (36). However, in breast cancer ST6GalNAc-I RNA is correlated with STn expression (17). STn is expressed in approximately one-quarter to one-third of breast carcinomas and is associated with metastatic competence, poor response to chemotherapy and poor prognosis (37-41). STn expression has also been reported in several other carcinomas including gastric, pancreatic, colorectal, ovarian and cervical (42-47). STn is often associated with lymph node involvement, distant metastasis, and a decreased survival of patients with gastric (48-51) or colorectal cancer (52-54). However, the molecular mechanisms through which STn enhances the malignant phenotype have yet to be elucidated.

In this study, the expression profile of ppGalNAc-T6 and ST6GalNAc-I was assessed in a cohort of women with breast cancer. Transcript levels were evaluated against established pathological parameters and clinical outcome over a 10 year follow-up period.

Table I. *Clinical and pathological data.*

| Parameter | Number |
|---|---|
| Node status | |
| Node positive | 54 |
| Node negative | 73 |
| Tumour grade | |
| 1 | 24 |
| 2 | 43 |
| 3 | 58 |
| Tumour type | |
| Ductal | 98 |
| Lobular | 14 |
| Medullary | 2 |
| Tubular | 2 |
| Mucinous | 4 |
| Others | 7 |
| TNM staging | |
| 1 | 70 |
| 2 | 40 |
| 3 | 7 |
| 4 | 4 |
| Outcome | |
| Disease free | 90 |
| Alive with metastasis | 7 |
| With local recurrence | 5 |
| Died of breast cancer | 16 |
| Died of unrelated disease | 9 |

Note: Missing values reflect discarded/uninterpretable values.

## Patients and Methods

*Patients and samples.* Institutional guidelines, including ethical approval and informed consent, were followed. Breast cancer tissues (n=127) and normal background tissues (n=33) were collected immediately after excision during surgery and stored at –80˚C until use. A consultant pathologist examined haematoxylin and eosin stained frozen sections to verify the presence of tumour cells in the collected samples. Normal tissue was derived from the background breast parenchyma of breast cancer patients within the study group. Medical notes and histology reports were used to extract the clinico-

Table II. *Primers*.

| | |
|---|---|
| Primers for ppGalNAc-T6 | |
| ACTGAACCTGACCGTACAGAAATGTCACCGAAGGATT | GALNT6Zr1 |
| GGATGGACAGCTACAAGAAG | GALNT6F1 |
| ATGTTTGTTCCTGACCTGAC | GALNT6F2 |
| ACTGAACCTGACCGTACAGTACATGATGAGGGGCTTC | GALNT6Zr2 |
| Primers for ST6GalNAc-I | |
| ACTGAACCTGACCGTACATAAAGCCGTAGAAGGATGTC | ST6GalZr1 |
| GCCAGGAGATAGACAGTCAC | ST6GalF1 |
| ACCCAGACTTTCTCCGATAC | ST6GalF2 |
| ACTGAACCTGACCGTACAGGCGGTATATCCTCCAGT | ST6GalZr2 |
| Primers for GAPDH | |
| CTGAGTACGTCGTGGAGTC | GAPDHF2 |
| ACTGAACCTGACCGTACACAGAGATGATGACCCTTTTG | GAPDHZr2 |
| Primers for beta-actin | |
| ATGATATCGCCGCGCTCGTC | |
| CGCTCGGTGAGGATCTTCA | |

pathological data (Table I). A customized database was established to record the data.

*Materials*. RNA extraction kits and reverse transcription kits were obtained from Sigma-Aldrich Ltd (Poole, Dorset, England, UK). The PCR primers were designed using Beacon Designer (Palo Alto, CA, USA) and synthesized by Sigma-Aldrich. Custom made hot-start Master mix for quantitative PCR was obtained from Abgene (Surrey, England, UK) (55, 56).

*Tissue processing, RNA extraction and cDNA synthesis*. Frozen sections of tissue were cut at a thickness of 5-10 mm and kept for routine histological analysis. An additional 15-20 sections were mixed and homogenized using a hand-held homogenizer in ice-cold RNA extraction solution. The concentration of RNA was determined using UV spectrophotometry. Reverse transcription was carried out using a reverse transcription kit with an anchored olig (dT) primer supplied by Abgene, using 1 mg of total RNA in a 96-well plate. The quality of cDNA was verified using β-actin primers (Table II).

*Quantitative analysis of glycosyltransferases*. The level of ppGalNAc-T6 and ST6GalNAc-I transcripts from the above prepared DNA were determined using real-time quantitative PCR based on the Amplifluor technology, modified from a method reported previously (56). The PCR primers were designed using Beacon Designer software, but to the reverse primer an additional sequence, known as the Z sequence (5'-ACTGAACCTGACCGTACA-3') which is complementary to the universal Z probe (Intergen Inc., Oxford, UK) was added. The product expands one intron. The primers used for each glycosyltransferase are detailed in Table II. The reaction was carried out using Hotstart Q-master mix (Abgene), 10 pmol of specific forward primer, 1 pmol reverse primer which had the Z sequence, 10 pmol of FAM (fluorogenic reporter dye, carboxyfluorescein) tagged probe (Intergen Inc.), and cDNA from 50 ng of RNA. The reaction was carried out using the IcyclerIQ (Bio-Rad Ltd, Hemel Hempstead, England, UK), which is equipped with an optic unit that allows real-time detection of 96 reactions, under the following conditions: 94˚C for 12 min and 50 cycles of 94˚C for 15 sec, 55˚C for 40 sec, and 72˚C for 20 sec. The levels of the transcript were generated from a standard that was simultaneously amplified with the samples. The levels of ppGalNAc-T6 and ST6GalNAc-I expression were then normalized against the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was already quantified in these specimens, to correct for varying amounts of epithelial tissue between samples (57). The primers used for GAPDH are detailed in Table II. With every PCR run, a negative control without a template and a known cDNA reference sample as a positive control, were included.

*Statistical analysis*. The Mann-Whitney *U*-test and two-sample *t*-test were used for statistical analysis. The ppGalNAc-T6 and ST6GalNAc-I transcript levels within the breast cancer specimens were compared to normal background tissues and analyzed against conventional pathological parameters and clinical outcome over a 10 year follow-up period. In each case the true copy number was used for statistical analysis and hence the samples were not classified as positive or negative. The statistical analysis was carried out using Minitab version 14.1 (Minitab Ltd. Coventry, England, UK) using a custom written macro (Stat 2005.mtw). For purposes of the Kaplan-Meier survival analysis, the samples were divided arbitrarily into two groups, 'high transcript level' or 'low transcript level', for each glycosyltransferase enzyme. The cut-off was guided by the Nottingham Prognostic Index (NPI) value, with which the value of the moderate prognostic group was used as the dividing line at the start of the test. Survival analysis was performed using SPSS version 12.0.1 (SPSS Inc. Chicago, IL, USA).

## Results

*ppGalNAc-T6*. The ppGalNAc-T6 expression profiles were normalised against GAPDH (Table III). ppGalNAc-T6 was found to be expressed in both normal/benign breast tissue and breast cancer specimens. Significantly higher levels were

Table III. *Summary of expression profiles for the overall cohort, followed by subgroup analysis for tumour specimens and benign specimens. Values represent the true copy number of mRNA transcripts (normalised against GAPDH) and are expressed as mean (range, median).*

| | Overall | Tumour | Benign |
|---|---|---|---|
| ppGalNAc-T6 | 7.68 (0-353.40, 0) | 9.98 (0-353.40, 0) | 0.0666 (0-1.792, 0) |
| ST6GalNAc-I | 1656 (0-76648, 15) | 1227 (0-31742, 14) | 3257 (0-76648, 11) |

found in the breast cancer specimens compared to the background tissue (mean copy number=10.0 *vs.* 0.067, $p$=0.015). Although there was a trend for levels to increase with NPI and TNM stage and those who died from breast cancer, this did not reach statistical significance.

In addition, a significant association with ductal type was found (mean copy number=9.8 *vs.* 0.050, $p$=0.041). However, no relationship with tumour grade or oestrogen receptor (ER) status was observed.

The overall survival curve for women with tumours which were classified as having 'high levels' of ppGalNAc-T6 transcript was not found to differ significantly from that of their 'low level' counterparts, Figure 1 ($p$=0.38).

*ST6GalNAc-I.* The ST6GalNAc-I expression profiles were normalised against GAPDH (Table III). ST6GalNAc-I was found to be expressed in both normal/benign breast tissue and breast cancer specimens. Although higher in the former, this did not reach statistical significance. Higher levels were found to be associated with better prognosis, this reached statistical significance when comparing patients who remained disease free to those with distant recurrence (mean copy number=1640 *vs.* 0.111, $p$=0.0096). The relationship approached significance when comparing NPI 2 to NPI 3 (mean copy number=2388 *vs.* 148, $p$=0.058) and disease free patients to non-disease free patients (mean copy number=1640 *vs.* 381, $p$=0.052) or those who died of breast cancer (mean copy number=1640 *vs.* 398, $p$=0.060).

In addition, a significant association with ductal type was found (mean copy number=1397 *vs.* 51, $p$=0.014). However, no relationship with tumour grade or ER status was observed. Within the ductal carcinoma subgroup, high expression remained associated with favourable prognosis and reached statistical significance when comparing patients who remained disease free to those with distant recurrence (mean copy number=1855 *vs.* 0.138, $p$=0.018).

The overall survival curve for women with tumours which were classified as having 'high levels' of ST6GalNAc-I transcript was not found to differ significantly from that of their 'low level' counterparts, Figure 2 ($p$=0.8).

## Discussion

In the present study, ppGalNAc-T6 expression was identified in both normal/benign breast tissue and breast cancer specimens. However, significantly higher levels were found in the breast cancer specimens compared to the background tissue. The specificity of ppGalNAc-T6 for breast cancer has been demonstrated by Freire *et al.* (25), who identified expression in all 3 human breast cancer cell lines evaluated and 88% of primary breast cancers. These findings have been confirmed by Brooks *et al.* (26) who also identified an increased range of ppGalNAc-T's in malignant cell lines, compared to their 'normal'/benign counterparts. Berois *et al.* (12), demonstrated ppGalNAc-T6 expression in 81% of a series of breast carcinomas and 91% of ductal carcinoma in-situ (DCIS), in contrast to only 20% of benign lesions and none of the five normal breast tissue samples. Furthermore, a significant association with T1 stage was found, suggesting that up-regulation may represent an early event. Expression was shown to continue in aggressive and metastatic lesions, however, no correlation with nodal status or histological grade was identified (12). ppGalNAc-T6 has also been demonstrated in bone marrow aspirates from breast cancer patients, where expression was significantly associated with early recurrence (25). Hence, ppGalNAc-T6 could offer prognostic utility in addition to conventional markers. In this study, although the ppGalNAc-T6 levels tended to increase with NPI and TNM stage and in those who died from breast cancer, no significant differences were identified in the overall survival curve for women with tumours which were classified as having 'high levels' of ppGalNAc-T6 transcript compared to their 'low level' counterparts, Figure 1.

In the present study, ST6GalNAc-I expression was found to be associated with better prognosis, this reached statistical significance when comparing patients who remained disease free to those with distant recurrence and approached significance when comparing NPI 2 to NPI 3 and disease free patients to non-disease free patients or those who died of breast cancer. However, the relationship between ST6GalNAc-I and STn expression could not be examined as the latter was not evaluated. At first glance, the observed relationship between ST6GalNAc-I expression and patient outcome did not appear to be consistent with the established association between STn expression and poor patient prognosis. ST6GalNAc-I has been demonstrated to be the major STn synthase *in vitro* and evidence supports an equivalent role *in vivo* (31). However, some authors have failed to find an association between ST6GalNAc I and STn expression levels. Despite the fact that STn has been found to be expressed in colonic cancer, rather than normal colonic tissue, the level of ST6GalNAc-I was comparable between the two (58, 59). This suggests that the relationship between ST6GalNAc-I and STn expression may be complicated by

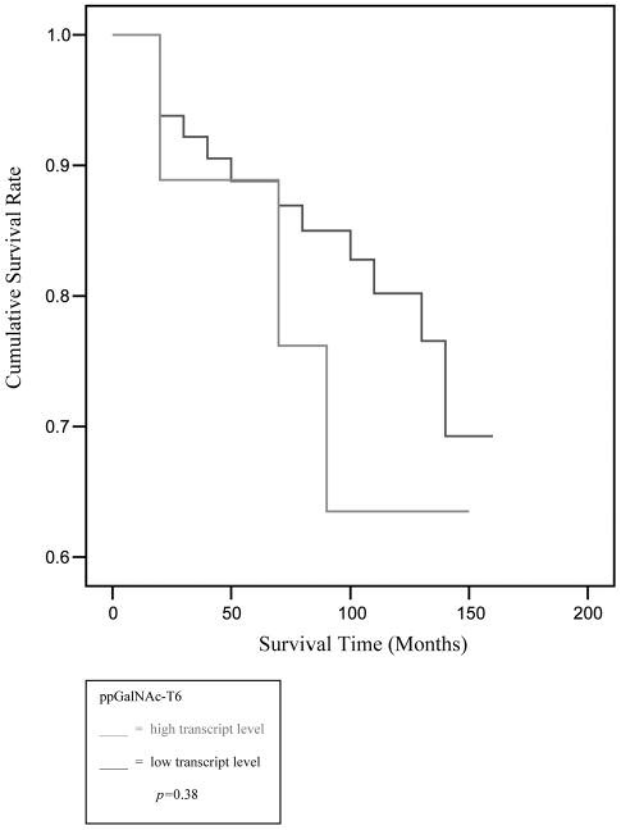


Figure 1. *Overall survival curve for ppGalNAc-T6.*

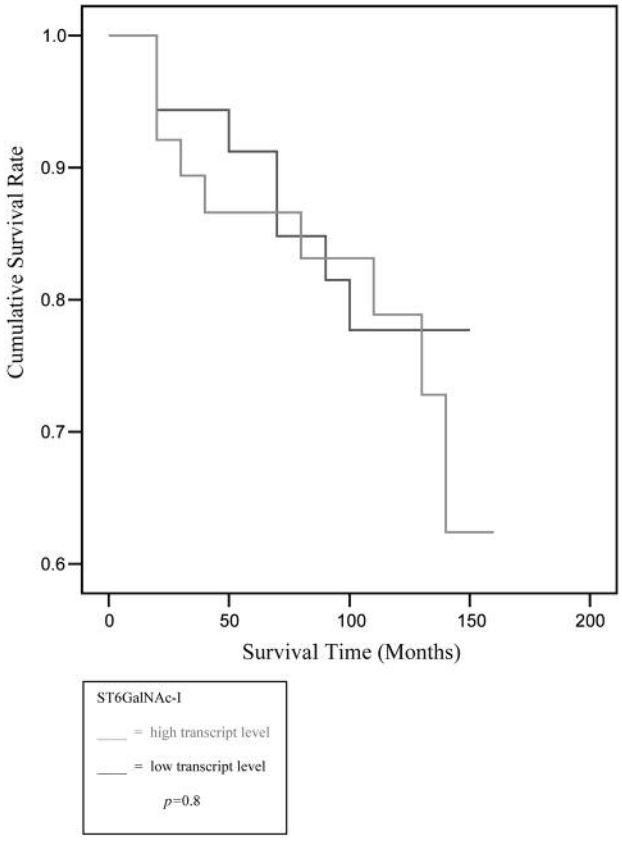


Figure 2. *Overall survival curve for ST6GalNAc-I.*

other mechanisms. In keeping with this, Tn and STn expression in cervical cancer specimens, colon cancer and melanoma derived cell lines have recently been found to be associated with loss-of-function somatic mutations in the Cosmc gene. This single exon gene encodes a molecular chaperone required for formation of the active T-synthase (42). Hence, specific genetic mutations within glycosylation pathways can directly influence expression of these antigens across a range of different neoplasms.

Breast cancer cell lines transfected with ST6GalNAc-I show significantly altered patterns of O-glycosylation and exhibit enhanced tumourigenicity, in terms of decreased adhesion, increased migration and increased growth (33, 58, 61-63). Similarly, STn expression is associated with decreased cell adhesion on extracellular matrix components and increased cell migration (33). STn bearing mucins may also interfere with cancer cell recognition by the immune system and natural killer cell function, perhaps protecting blood borne metastatic cells from degradation (64). Furthermore, anti-STn-mAb/STn-bearing protein immunocomplexes enhance vascular endothelial growth factor secretion by tumour-infiltrating macrophages, improving angiogenesis (65). STn expression has also been associated with the invasiveness of ovarian carcinomas, with enhanced expression in the invasive front (66-68). Davidson *et al*. also found that the expression of STn of distant metastases appeared to be significantly lower than that of the primary ovarian tumour, suggesting that such changes may be transient and play a greater role at particular stages of the metastatic pathway, for example, facilitating the dissociation of cancer cells from the primary tumours.

STn expression has been associated with poor prognosis in a range of solid human carcinomas and appears to identify patients that may be relatively refractory to conventional treatment (52, 53, 69). The selective expression of STn has been considered for its therapeutic utility (70-74). Cancer vaccine studies have also identified STn as a potential target in breast and ovarian carcinomas (75, 76). In addition to the differential expression of STn between normal tissue and carcinomas, altered expression has also been observed in pre-malignant lesions of the gastrointestinal tract, such as intestinal metaplasia (50), adenomatous polyps (77) and chronic ulcerative colitis (78). Hence, STn is likely to have an important role to play in the development and progression of the malignant phenotype.

Limitations of the present study included the use of background parenchyma from breast cancer patients to provide 'normal tissue' for comparison. Ideally, such material should be derived from patients without breast cancer in order to avoid any 'field change' which may exist within cancer bearing tissues. Although the sample size and follow-up period were substantial, it is possible that a larger cohort may have influenced several results which approached, but failed to reach, statistical significance. Finally, in addition to the measurement of mRNA transcript levels, quantitative analysis of enzyme, Tn and STn expression should be undertaken to ensure concordance.

## Conclusion

The expression of ppGalNAc-T6 is significantly higher in breast carcinomas compared to 'normal'/benign breast tissue samples and ST6GalNAc-I expression is significantly associated with better prognosis. Further studies are required to elucidate their contribution the development and progression of the malignant phenotype.

## References


1 Hanisch FG: O-glycosylation of the mucin type. Biol Chem *382(2)*: 143-149, 2001.

2 Brockhausen I: Mucin-type O-glycans in human colon and breast cancer: glycodynamics and functions. EMBO Rep *7(6)*: 599-604, 2006.

3 Hakomori S: Carbohydrate-to-carbohydrate interaction in basic cell biology: a brief overview. Arch Biochem Biophys *426(2)*: 173-181, 2004.

4 Varki A and Angata T: Siglecs – the major sub-family of I-type lectins. Glycobiology *16*: 1R-27R, 2006.

5 Gerloni M, Castiglioni P and Zanetti M: The cooperation between two CD4 T-cells induces tumor protective immunity in MUC.1 transgenic mice. J Immunol *175*: 6551-6559, 2005.

6 Crocker PR: Siglecs in innate immunity. Curr Opin Pharmacol *5*: 431-437, 2005.

7 Baldus SE, Engelmann K and Hanisch FG: MUC1 and the MUCs: a family of human mucins with impact in cancer biology. Crit Rev Clin Lab Sci *41*: 189-231, 2004.

8 Onami TM, Harrington LE, Williams MA, Galvan M, Larsen CP, Pearson TC, Manjunath N, Baum LG, Pearce BD and Ahmed R: Dynamic regulation of T cell immunity by CD43. J Immunol *168*: 6022-6031, 2002.

9 Hollingsworth MA and Swanson BJ: Mucins in cancer: protection and control of the cell surface. Nat Rev Cancer *4*: 45-60, 2004.

10 Kim Y and Varki A: Perspectives on the significance of altered glycosylation of glycoproteins in cancer. Glycoconjugate J *14*: 569-576, 1997.

11 Dabelsteen E: Cell surface carbohydrates as a prognostic marker in human carcinomas. J Pathol *179*: 359-369, 1996.

12 Berois N, Mazal D, Ubillos L, Trajtenberg F, Nicolas A, Sastre-Garau X, Magdelenat H and Osinaga E: UDP-N-acetyl-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase-6 as a new immunohistochemical breast cancer marker. J Histochem Cytochem *54(3)*: 317-328, 2006.

13 Ten Hagen KG, Fritz TA and Tabak LA: All in the family: the UDPGalNAc: polypeptide N-acetylgalactosaminyltransferases. Glycobiology *13*: 1R-16R, 2003.

14 Röttger S, White J, Wandall HH, Olivo JC, Stark A, Bennett EP, Whitehouse C, Berger EG, Clausen H and Nilsson T: Localization of three human polypeptide GalNAc-transferases in HeLa cells suggests initiation of O-linked glycosylation throughout the Golgi apparatus. J Cell Sci *111(1)*: 45-60, 1998.

15 Brockhausen I: Pathways of O-glycan biosynthesis in cancer cells. Biochim Biophys Acta *1473*: 67-95, 1999.

16 Brockhausen I: Glycodynamics of mucin biosynthesis in gastrointestinal tumour cells. Adv Exp Med Biol *535*: 163-188, 2003.

17 Sewell R, Bäckström M, Dalziel M, Gschmeissner S, Karlsson H, Noll T, Gätgens J, Clausen H, Hansson GC, Burchell J and Taylor-Papadimitriou J: The ST6GalNAc-I sialyltransferase localizes throughout the Golgi and is responsible for the synthesis of the tumor-associated sialyl-Tn O-glycan in human breast cancer. J Biol Chem *281(6)*: 3586-3594, 2006.

18 Brooks SA, Carter TM, Royle L, Harvey DJ, Fry SA, Kinch C, Dwek RA and Rudd PM: Altered glycosylation of proteins in cancer: what is the potential for new anti-tumour strategies. Anticancer Agents Med Chem *8(1)*: 2-21, 2008.

19 Springer GF, Desai PR, Spencer BD, Tegtmeyer H, Carlstadt SC and Scanlon EF: T/Tn antigen vaccine is effective and safe in preventing recurrence of advanced breast carcinoma. Cancer Detect Prevent *19*: 374-380, 1995.

20 Springer GF: Immunoreactive T and Tn epitopes in cancer diagnosis, prognosis, and immunotherapy. J Mol Med *75*: 594-602, 1997.

21 Lo-Man R, Vichier-Guerre S, Bay S, Deriaud E, Cantacuzene D and Leclerc C: Anti-tumor immunity provided by a synthetic multiple antigenic glycopeptide displaying a tri-Tn glycotope. J Immunol *166*: 2849-2854, 2001.

22 Bennett EP, Hassan H, Mandel U, Hollingsworth MA, Akisawa N, Ikematsu Y *et al*: Cloning and characterization of a close homologue of human UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase- T3, designated GalNAc-T6. Evidence for genetic but not functional redundancy. J Biol Chem *274*: 25362-25370, 1999.

23 Wandall HH, Dabelsteen S, Sorensen JA, Krogdahl A, Mandel U and Dabelsteen E: Molecular basis for the presence of glycosylated onco-foetal fibronectin in oral carcinomas: the production of glycosylated oncofoetal fibronectin by carcinoma cells. Oral Oncol *43*: 301-309, 2007.

24 Nomoto M, Izumi H, Ise T, Kato K, Takano H, Nagatani G, Shibao K, Ohta R, Imamura T, Kuwano M, Matsuo K, Yamada Y *et al*: Structural basis for the regulation of UDP-N-acetyl-a-D-galactosamine: polypeptide N-acetylgalactosaminyl transferase-3 gene expression in adenocarcinoma cells. Cancer Res *59*: 6214-6222, 1999.

25 Freire T, Berois N, Sóñora C, Varangot M, Barrios E and Osinaga E: UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 6 (ppGalNAc-T6) mRNA as a potential new marker for detection of bone marrow-disseminated breast cancer cells. Int J Cancer *119(6)*: 1383-1388, 2006.

26 Brooks SA, Carter TM, Bennett EP, Clausen H and Mandel U: Immunolocalisation of members of the polypeptide N-acetylgalactosaminyl transferase (ppGalNAc-T) family is consistent with biologically relevant altered cell surface glycosylation in breast cancer. Acta Histochem *109(4)*: 273-284, 2007.

27 Burchell JM, Mungul A and Taylor-Papadimitriou J: O-linked glycosylation in the mammary gland: changes that occur during malignancy. J Mammary Gland Biol Neoplasia *6*: 355-364, 2001.
28 Lloyd KO, Burchell J, Kudryashov V, Yin BW and Taylor-Papadimitriou J: Comparison of O-linked carbohydrate chains in MUC-1 mucin from normal breast epithelial cell lines and breast carcinoma cell lines. Demonstration of simpler and fewer glycan chains in tumor cells. J Biol Chem *271(52)*: 33325-33334, 1996.
29 Paulson JC and Colley KJ: Glycosyltransferases. Structure, localization, and control of cell type-specific glycosylation. J Biol Chem *264*: 17615-17618, 1989.
30 Ikehara Y, Kojima N, Kurosawa N, Kudo T, Kono M, Nishihara S, Issiki S, Morozumi K, Itzkowitz S, Tsuda T, Nishimura SI, Tsuji S and Narimatsu H: Cloning and expression of a human gene encoding an N-acetylgalactosamine-alpha2,6-sialyltransferase (ST6GalNAc I): a candidate for synthesis of cancer-associated sialyl-Tn antigens. Glycobiology *9(11)*: 1213-1224, 1999.
31 Marcos NT, Pinho S, Grandela C, Cruz A, Samyn-Petit B, Harduin-Lepers A, Almeida R, Silva F, Morais V, Costa J, Kihlberg J, Clausen H and Reis CA: Role of the human ST6GalNAc-I and ST6GalNAc-II in the synthesis of the cancer-associated sialyl-Tn antigen. Cancer Res *64(19)*: 7050-7057, 2004.
32 Burdick MD, Harris A, Reid CJ, Iwamura T and Hollingsworth MA: Oligosaccharides expressed on MUC1 produced by pancreatic and colon tumor cell lines. J Biol Chem *272*: 24198-24202, 1997.
33 Julien S, Lagadec C, Krzewinski-Recchi MA, Courtand G, Le Bourhis X and Delannoy P: Stable expression of sialyl-Tn antigen in T47-D cells induces a decrease of cell adhesion and an increase of cell migration. Breast Cancer Res Treat Mar *90(1)*: 77-84, 2005.
34 Singh R, Campbell BJ, Yu LG, Fernig DG, Milton JD, Goodlad RA, FitzGerald AJ and Rhodes JM: Cell surface expressed Thomsen-Friedenreich antigen in colon cancer is predominantly carried on high molecular weight splice variants of CD44. Glycobiology *11*: 587-592, 2001.
35 Clement M, Rocher J, Loirand G and Le Pendu J: Expression of sialyl-Tn epitopes on beta1 integrin alters epithelial cell phenotype, proliferation and haptotaxis. J Cell Sci *117*: 5059-5069, 2004.
36 Cao Y, Stosiek P, Springer GF and Karsten U: Thomsen-Friedenreich-related carbohydrate antigens in normal adult human tissues: a systematic and comparative study. Histochem Cell Biol *106(2)*: 197-207, 1996.
37 Imai J, Ghazizadeh M, Naito Z and Asano G: Immunohistochemical expression of T, Tn and sialyl-Tn antigens and clinical outcome in human breast carcinoma. Anticancer Res *21(2B)*: 1327-1334, 2001.
38 Miles DW, Happerfield LC, Smith P, Gillibrand R, Bobrow LG, Gregory WM and Rubens RD: Expression of sialyl-Tn predicts the effect of adjuvant chemotherapy in node-positive breast cancer. Br J Cancer *70(6)*: 1272-1275, 1994.
39 Leivonen M, Nordling S, Lundin J, von Boguslawski K and Haglund C: STn and prognosis in breast cancer. Oncology *61(4)*: 299-305, 2001.
40 Schmitt FC, Figueiredo P and Lacerda M: Simple mucin-type carbohydrate antigens (T, sialosyl-T, Tn and sialosyl-Tn) in breast carcinogenesis. Virchows Arch *427*: 251-258, 1995.
41 Soares R, Marinho A and Schmitt F: Expression of sialyl-Tn in breast cancer. Correlation with prognostic parameters. Pathol Res Pract *192*: 1181-1186, 1996.
42 Ju T, Lanneau GS, Gautam T, Wang Y, Xia B, Stowell SR, Willard MT, Wang W, Xia JY, Zuna RE, Laszik Z, Benbrook DM, Hanigan MH and Cummings RD: Human tumor antigens Tn and sialyl Tn arise from mutations in Cosmc. Cancer Res *68(6)*: 1636-1646, 2008.
43 Julien S and Delannoy P: Sialyl-Tn antigen in cancer: from diagnosis to therapy. In: Recent Research Developments in Cancer. Transworld Research Network, Kerala, India, pp. 185-199, 2003.
44 Hakomori S: Tumor-associated carbohydrate antigens defining tumor malignancy: basis for development of anti-cancer vaccines. Adv Exp Med Biol *491*: 369-402, 2001.
45 Kobayashi H, Terao T and Kawashima Y: Serum sialyl Tn as an independent predictor of poor prognosis in patients with epithelial ovarian cancer. J Clin Oncol *10*: 95-101, 1992.
46 Kim GE, Bae HI, Park HU *et al*: Aberrant expression of MUC5AC and MUC6 gastric mucins and sialyl Tn antigen in intraepithelial neoplasms of the pancreas. Gastroenterology *123*: 1052-1060, 2002.
47 Numa F, Tsunaga N, Michioka T, Nawata S, Ogata H and Kato H: Tissue expression of Sialyl Tn antigen in gynecologic tumors. J Obstet Gynaecol *21*: 385-389, 1995.
48 Imada T, Rino, Y, Hatori S, Takahashi M, Amano T, Kondo J and Suda T: Sialyl Tn antigen expression is associated with the prognosis of patients with advanced colorectal cancer. Hepatogastroenterology *46*: 208-214, 1999.
49 Takano Y, Teranishi Y, Terashima S, Motoki R and Kawaguchi T: Lymph node metastasis-related carbohydrate epitopes of gastric cancer with submucosal invasion. Surg Today *30*: 1073-1082, 2000.
50 David L, Nesland JM, Clausen H, Carneiro F and Sobrinho-Simoes M: Simple mucin type carbohydrate antigens (Tn, sialosyl-Tn and T) in gastric mucosa, carcinomas and metastases. APMIS Suppl *27*: 162-172, 1992.
51 Victorzon M, Nordling S, Nilsson O, Roberts PJ and Haglund C: Sialyl Tn antigen is an independent predictor of outcome in patients with gastric cancer. Int J Cancer *65*: 295-300, 1996.
52 Nakagoe T, Sawai T, Tsuji T, Jibiki M, Nanashima A, Yamaguchi H, Kurosaki N, Yasutake T and Ayabe H: Circulating sialyl Lewis (x), sialyl Lewis (a), and sialyl Tn antigens in colorectal cancer patients: multivariate analysis of predictive factors for serum antigen levels. J Gastroenterol *36*: 166-172, 2001.
53 Nakagoe T, Sawai T, Tsuji T, Jibiki M, Nanashima A, Yamaguchi H, Yasutake T, Ayabe H, Arisawa K and Ishikawa H: Preoperative serum levels of sialyl Tn antigen predict liver metastasis and poor prognosis in patients with gastric cancer. Eur J Surg Oncol *27(8)*: 731-739, 2001.
54 Itzkowitz SH, Bloom EJ, Kokal WA, Modin G, Hakomori S and Kim YS: Sialosyl-Tn. A novel mucin antigen associated with prognosis in colorectal cancer patients. Cancer (Phila) *66*: 1960-1966, 1990.
55 Jiang WG, Douglas-Jones A and Mansel RE: Level of expression of PPAR-gamma and its co-activator (PPAR-GCA) in human breast cancer. Int J Cancer *106*: 752-757, 2003.
56 Jiang WG, Watkins G, Lane J *et al*: Prognostic value of Rho familty and and rho-GDIs in breast cancer. Clin Cancer Res *9*: 6432-6440, 2003.

57 Jiang WG,Watkins G, Fodstad O *et al*: Differential expression of the CCN family members Cyr61 from CTGF and Nov in human breast cancer. Endocr Relat Cancer *11*: 781-791, 2004.

58 Brockhausen I, Yang J, Lehotay M, Ogata S and Itzkowitz S: Pathways of mucin O-glycosylation in normal and malignant rat colonic epithelial cells reveal a mechanism for cancer-associated Sialyl-Tn antigen expression. Biol Chem *382*: 219-232, 2001.

59 Vázquez-Martín C, Cuevas E, Gil-Martín E and Fernández-Briera A: Correlation analysis between tumor-associated antigen sialyl-Tn expression and ST6GalNAc I activity in human colon adenocarcinoma. Oncology *67(2)*: 159-165, 2004.

60 Kinney AY, Sahin A, Vernon SW, Frankowski RF, Annegers JF, Hortobagyi GN, Buzdar AU, Frye DK and Dhingra K: The prognostic significance of sialyl-Tn antigen in women treated with breast carcinoma treated with adjuvant chemotherapy. Cancer *80(12)*: 2240-2249, 1997.

61 Julien S, Adriaenssens E, Ottenberg K, Furlan A, Courtand G, Vercoutter-Edouart AS, Hanisch FG, Delannoy P and Le Bourhis X: ST6GalNAc I expression in MDA-MB-231 breast cancer cells greatly modifies their O-glycosylation pattern and enhances their tumourigenicity. Glycobiology *16(1)*: 54-64, 2006.

62 Julien S, Krzewinski-Recchi MA, Harduin-Lepers A, Gouyer V, Huet G, Le Bourhis X and Delannoy P: Expression of sialyl-Tn antigen in breast cancer cells transfected with the human CMP-Neu5Ac: GalNAc alpha2,6-sialyltransferase (ST6GalNac I) cDNA. Glycoconjugate J *18(11-12)*: 883-893, 2001.

63 Muller S and Hanisch FG: Recombinant MUC1 probe authentically reflects cell-specific O-glycosylation profiles of endogenous breast cancer mucin: high-density and prevalent core2-based glycosylation. J Biol Chem *277*: 26103-26112, 2002.

64 Ogata S, Maimonis PJ and Itzkowitz SH: Mucins bearing the cancer-associated sialosyl-Tn antigen mediate inhibition of natural killer cell cytotoxicity. Cancer Res *52*: 4741-4746, 1992.

65 Barbera-Guillem E, Nyhus JK, Wolford CC, Friece CR and Sampsel JW: Vascular endothelial growth factor secretion by tumor-infiltrating macrophages essentially supports tumor angiogenesis, and IgG immune complexes potentiate the process. Cancer Res *62*: 7042-7049, 2002.

66 Davidson B, Gotlieb WH, Ben-Baruch G, Kopolovic J, Goldberg I, Nesland JM, Berner A, Bjamer A and Bryne M: Expression of carbohydrate antigens in advanced-stage ovarian carcinomas and their metastases – a clinicopathologic study. Gynecol Oncol *77*: 35-43, 2000.

67 Davidson B, Berner A, Nesland JM, Risberg B, Kristensen GB, Trope CG and Bryne M: Carbohydrate antigen expression in primary tumors, metastatic lesions, and serous effusions from patients diagnosed with epithelial ovarian carcinoma: evidence of up-regulated Tn and Sialyl Tn antigen expression in effusions. Hum Pathol *31*: 1081-1087, 2000.

68 Wang PH, Lee WL, Juang CM *et al*: Altered mRNA expressions of sialyltransferases in ovarian cancers. Gynecol Oncol *99*: 631-639, 2005.

69 Nakagoe T, Sawai T, Tuji T, Jibiki M, Nanashima A, Yamaguchi H, Yasutake T, Ayabe H, Matuo T and Tagawa Y: Prognostic value of expression of sialosyl-Tn antigen in colorectal carcinoma and transitional mucosa. Dig Dis Sci *47(2)*: 322-330, 2002.

70 Miles D and Papazisis K: Rationale for the clinical development of STn-KLH (Theratope) and anti-MUC-1 vaccines in breast cancer. Clin Breast Cancer *3(Suppl 4)*: S134-138, 2003.

71 Miles DW, Towlson KE, Graham R, Reddish M, Longenecker BM, Taylor-Papadimitriou J and Rubens RD: A randomised phase II study of sialyl-Tn and DETOX-B adjuvant with or without cyclophosphamide pretreatment for the active specific immunotherapy of breast cancer. Br J Cancer *74(8)*: 1292-1296, 1996.

72 Miles DW, Happerfield LC, Smith P, Gillibrand R, Bobrow LG, Gregory WM and Rubens RD: Expression of sialyl-Tn predicts the effect of adjuvant chemotherapy in node-positive breast cancer. Br J Cancer *70(6)*: 1272-1275, 1994.

73 MacLean GD, Reddish M, Koganty RR, Wong T, Gandhi S, Smolenski M, Samuel J, Nabholtz JM and Longenecker BM: Immunization of breast cancer patients using a synthetic sialyl-Tn glycoconjugate plus Detox adjuvant. Cancer Immunol Immunother *36(4)*: 215-222, 1993.

74 MacLean GD, Reddish MA, Koganty RR and Longenecker BM: Antibodies against mucin-associated sialyl-Tn epitopes correlate with survival of metastatic adenocarcinoma patients undergoing active specific immunotherapy with synthetic STn vaccine. J Immunother Emphasis Tumor Immunol *19(1)*: 59-68, 1996.

75 Holmberg LA, Oparin DV, Gooley T and Sandmaier BM: The role of cancer vaccines following autologous stem cell rescue in breast and ovarian cancer patients: experience with the STn-KLH vaccine (Theratope(R)). Clin Breast Cancer *3*: S144-151, 2003.

76 Galonic DP and Gin DY: Chemical glycosylation in the synthesis of glycoconjugate antitumour vaccines. Nature *446*: 1000-1007, 2007.

77 Itzkowitz SH, Bloom EJ, Lau TS and Kim YS: Mucin associated Tn and sialosyl-Tn antigen expression in colorectal polyps. Gut *33*: 518-523, 1992.

78 Karlen P, Young E, Brostrom O *et al*: Sialyl-Tn antigen as a marker of colon cancer risk in ulcerative colitis: relation to dysplasia and DNA aneuploidy. Gastroenterology *115*: 1395-1404, 1998.

*Received September 1, 2008*
*Accepted October 6, 2008*